DP Barcode: 434137 MRID No.: 49910309

DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT & ORAL LD_{50} TEST
' 141-1

1. **CHEMICAL:** Ipconazole PC Code No.: 125618

2. **TEST MATERIAL:** Ipconazole Purity: 98.4%

3. **CITATION**

Authors:	Taylor, K.
Title:	Ipconazole Acute Toxicity to Honey Bees (*Apis mellifera*)
Study Completion Date:	01/18/2005
Laboratory:	Huntingdon Life Sciences, Cambridgeshire, England, United Kingdom
Sponsor:	Kureha Chemical Industry Co., Ltd, Tokyo, Japan
Laboratory Report ID:	KRA 094
MRID No.:	49910309
DP Barcode:	434137

4. **REVIEWED BY:** Lauren Apakian, Staff Scientist, CDM/CSS-Dynamac JV

Signature: Lauren Apakian **Date:** 03/31/2017

APPROVED BY: Elizabeth Krupka, Staff Scientist, CDM/CSS-Dynamac JV

Signature: Elizabeth Krupka **Date:** 04/26/2017

5. **APPROVED BY:** Holly Rogers, Biologist, OPP/EFED/ERB5

Signature: Holly Rogers Digitally signed by HOLLY ROGERS Date: 2020.05.04 09:37:48 -04'00' **Date:** 05/04/2020

APPROVED BY: Hannah Yingling, Biologist, USEPA/OPP/EFED/ERB5

Signature: Hannah B. Yingling **Date:** 04/28/2020

6. **DISCLAIMER:** *This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.*

7. **STUDY PARAMETERS:**

Scientific Name of Test Organism: *Apis mellifera*

Age of Test Organism at Test Initiation: Adult worker bees
Type of Concentrations: Nominal
Definitive Test Duration: 48 hours

8. CONCLUSIONS:

The honey bee, *Apis mellifera*, was exposed to **Ipconazole** for 48 hours in the oral and the contact test. The oral and contact nominal concentrations were 100 µg ai/bee. The actual intake concentrations of Ipconazole in the oral toxicity test were not reported.

By 48 hours in the oral test, mortality was 0, 0, and 1.7% in the negative control, solvent control, and 100 µg ai/bee treatment group, respectively. At 4 hours, one bee in the 100 µg ai/bee treatment group displayed signs of lack of coordination but recovered by 24 hours. No other related sub-lethal effects were reported. By 48 hours in the contact test, mortality was 1.7, 0, and 0% in the negative control, solvent control, and 100 µg ai/bee treatment group, respectively. No related sub-lethal effects were reported.

The LD_{50} value for the oral test was >100 µg ai/bee. The LD_{50} value for the contact test was >100 µg ai/bee. As a result, Ipconazole is categorized as *practically non-toxic* to honey bees *on an acute contact and acute oral basis*.

This study is scientifically *sound* and *satisfies* EFED concerning the guideline requirements for a contact toxicity test with honeybees (Subdivision L, ' 141-1 or 850.3020). **This study is classified as *ACCEPTABLE*.**

Results - Oral Test:

LD_{50}: >100 µg ai/bee 95% C.I.: N/A
Probit Slope: N/A

Results - Contact Test:

LD_{50}: >100 µg ai/bee 95% C.I.: N/A
Probit Slope: N/A

9. ADEQUACY OF THE STUDY:

A. Classification: This study **is scientifically sound** and is classified as **acceptable**.

B. Rationale: Both tests met all of the required guidelines for limit tests, including mortality in both controls ≤ 10%, mortality of ≤ 1 bee in the limit concentration (100 µg ai/bee), and LD_{50} of the toxic standard is within the specified range.

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: The study author designed the study to comply with:

- OCSPP Guideline No. 850.3020: Honey Bee Acute Contact Toxicity;
- OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test;
- OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test; and
- EPPO No. 170: Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honeybees.

The reviewer assessed the study methods and results according to U.S. EPA Ecological Effects Test Guidelines OCSPP Guideline 850.3020: Honey Bee Acute Contact Toxicity Test and OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test. Deviations were noted:

1. As a limit study was conducted, LD_{50} values were estimated and 95% confidence limits could not be calculated.
2. In the oral test, the rates of consumption of treated and untreated diets were not reported/monitored.

These deviations did have an impact on the acceptability of this study.

11. SUBMISSION PURPOSE: To determine the effects on mortality and sub-lethal effects of Ipconazole on honeybee (*A. mellifera*) adults from acute [single dose] oral or contact exposure following the U.S. EPA OCSPP Guideline 850.3020 (Honey Bee Acute Contact Toxicity Test) and OECD Guideline 213 (OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test) for the purpose of pesticide registration.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	Adult worker bees
Supplier:	Fowlmere Apiaries, Hertfordshire, England, United Kingdom
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Wire mesh cage Size: 11 cm tall x 4.0 cm diameter
Lighting:	Darkness; observations made under subdued light
Temperature:	25 to 26°C
Relative humidity:	67 to 70%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	Yes. Ipconazole was administered both orally and dermally at nominal doses of 0.01, 0.1, 1, 10, and 100 µg ai/bee. This test indicated both the oral and contact LD_{50} values were >100 µg ai/bee.
Reference toxicant test?	Yes; Pestanal (AI: Dimethoate; 0.0324, 0.054, 0.09, 0.15, and 0.25 µg ai/bee)

Guideline Criteria	Reported Information
Method of administration:	Oral test: Ipconazole was dissolved in acetone and this resulting solution was mixed with the necessary amount of 50% sucrose solution to reach the test concentrations. Prior to test feeding, bees were starved for one hour and 35 minutes. 200 µL of solution were then offered in a glass tube to each cage of 10 bees. Contact test: Bees were lightly anesthetized with carbon dioxide and laid in a petri dish lined with filter paper. Ipconazole was dissolved in acetone and applied in 1 µL droplets to the dorsal thorax of each bee using a micro-applicator. Control bees were dosed with an equivalent volume of reverse osmosis water and acetone in the same manner.
Nominal doses:	Oral test and Contact Tests: 0 (controls) and 100 µg ai/bee
Controls: Negative control and/or diluent/solvent control	Oral test: Negative control – 50% sucrose solution; solvent control – 50% sucrose solution + acetone Contact test: Negative control – water; solvent control – acetone.
Number of colonies per group:	Oral test: 10 bees per replicate unit, with 6 replicates per group Contact test: 10 bees per replicate unit, with 6 replicates per group
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Oral test: Acetone Contact test: Acetone

Guideline Criteria	Reported Information
Feeding:	A 50% sucrose solution was offered *ad libitum*. Oral test: 50% aqueous sugar solution was provided in the test cages after dosing. Contact test: 50% aqueous sugar solution was provided in the test cages after dosing.
Observations period:	Oral test: 4, 24, and 48 hours Contact test: 4, 24, and 48 hours

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, Good Laboratory Practice (GLP) Standards, and Quality Assurance statements were provided. This study was conducted in compliance with GLP Standards as published by the UK GLP Regulations; EC Commission Directive 2004/10/EC; and OECD Principles of Good Laboratory Practice.
Control performance:	Oral test: Negative: 0% mortality Solvent: 0% mortality Contact test: Negative: 1.7% mortality Solvent: 0% mortality
Raw data included:	Yes
Signs of toxicity (if any) were described?	Yes

Mortality - Oral Test

Dosage µg ai/bee (actual intake)	No. of bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Negative Control	60	0	0
Solvent Control	60	0	0
100	60	1.7	1.7
Toxic Standard			
Water Control	30	0	0
Solvent Control	30	0	0
0.0324	30	0	0
0.054	30	0	0
0.09	30	0	0
0.15	30	10	10
0.25	30	80	80

Observations: At 4 hours, one bee in the 100 µg ai/bee treatment group displayed signs of incoordination, but recovered by 24 hours. No other observed sub-lethal effects were reported in this test.

Mortality - Contact Test

Dosage µg ai/bee	No. of bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Negative Control	60	1.7	1.7
Solvent Control	60	0	0
100	60	0	0
Toxic Standard			
Negative Control	30	3.3	6.7
Solvent Control	30	0	0
0.0324	30	0	3.3
0.054	30	3.3	3.3
0.09	30	3.3	3.3
0.15	30	0	6.7
0.25	30	46.7	63.3

Observations: No observed sub-lethal effects were reported in this test.

Statistical method: Because bee mortality in both the oral and acute contact tests did not reach 50% at the test concentrations, the LD_{50} was estimated. Therefore, no statistical methods were reported.

Reported Statistical Results - Oral Test:

LD_{50}: >100 µg ai/bee 95% C.I.: N/A
NOAEL: Not determined Probit Slope: N/A
LOAEL: Not determined

Reported Statistical Results - Contact Test:

LD_{50}: >100 µg ai/bee 95% C.I.: N/A
NOAEL: Not determined Probit Slope: N/A
LOAEL: Not determined

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: Toxicity values were estimated based on a lack of mortality. Data were entered into CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 10/20/2015. Two test entries were made in CETIS – one each for the contact and oral tests.

Results - Oral Test:

LD_{50}: >100 µg ai/bee 95% C.I.: N/A
Probit Slope: N/A

Results - Contact Test:

LD_{50}: >100 µg ai/bee 95% C.I.: N/A
Probit Slope: N/A

15. REVIEWER'S COMMENTS:

The reviewer's results agreed with those reported by the study authors.

In the contact test after 48 hours, cumulative mortality 1.7% in the water control and 0.0% in the acetone control and in the 100.0 µg ai/bee treatment level.

In the oral test after 48 hours, cumulative mortality was 0.0% in the water and acetone controls and 1.7% at 100 µg ai/bee treatment level.

The in-life phase of this study took place from August 12 to August 14, 2004.

16. REFERENCES:

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.

CETIS Analytical Report

Test Code: 49910309 contac | 00-3422-5983

OCSPP 850.3020 Acute Honey Bee Test **Huntingdon Life Sciences**

Analysis ID:	08-3142-5762	**Endpoint:**	48h Mortality Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Apr-17 13:14	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	08-7679-1144	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	12 Aug-04	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Reverse Osmosis Water
Ending Date:		**Species:**	Apis mellifera	**Brine:**	
Duration:	n/a	**Source:**	Fowlmere Apiaries	**Age:**	

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	100µg ai/bee passed 48h mortality rate	3.07%

Equal Variance t Two-Sample Test

Control	vs	Conc-µg ai/b	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		100	1	1.812	0.030	10	CDF	0.1704	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0008333	0.0008333	1	1	0.3409	Non-Significant Effect
Error	0.0083333	0.0008333	10			
Total	0.0091667		11			

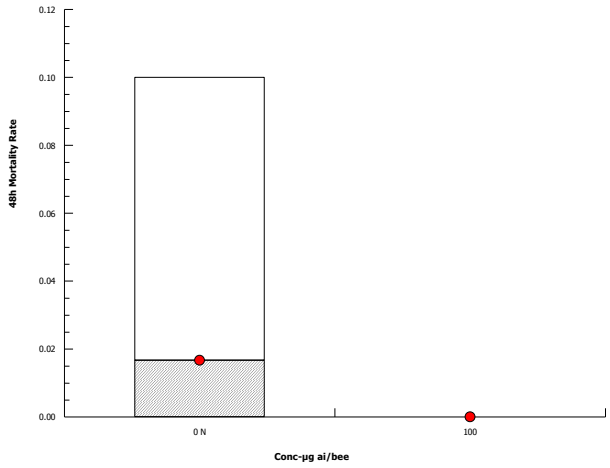
Distributional Tests

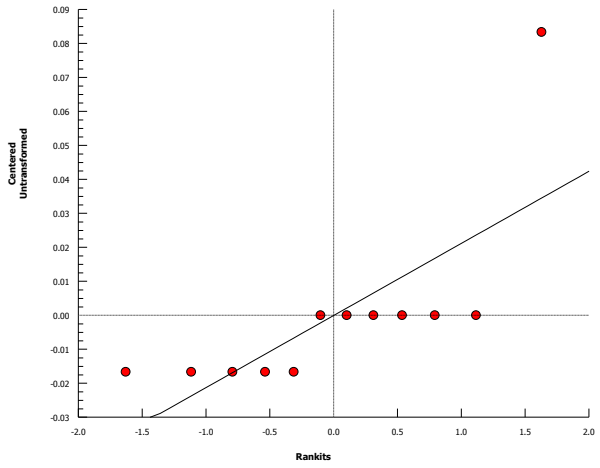
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Levene Equality of Variance Test	6.25	10.04	0.0314	Equal Variances
Variances	Mod Levene Equality of Variance Test	1	10.04	0.3409	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.5612	0.8025	5.2E-05	Non-Normal Distribution

48h Mortality Rate Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	6	0.0167	0.0000	0.0595	0.0000	0.0000	0.1000	0.0167	244.95%	0.00%
100		6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		-1.69%

Graphics





CETIS Summary Report

OCSPP 850.3020 Acute Honey Bee Test **Huntingdon Life Sciences**

Batch ID:	08-7679-1144	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	12 Aug-04	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Reverse Osmosis Water
Ending Date:		**Species:**	Apis mellifera	**Brine:**	
Duration:	NA	**Source:**	Fowlmere Apiaries	**Age:**	
Sample ID:	02-1544-3611	**Code:**	49910309	**Client:**	CDM Smith - E. Krupka
Sample Date:	12 Aug-04	**Material:**	Ipconazole	**Project:**	Fungicide
Receive Date:		**Source:**	Kureha Corporation		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 125618 MRID 49910309 48h Mortality Rate' endpoint… Error with Log-Normal (Probit) Model: The model requires two or more partial responses.

Sample Note: PC Code 125618 MRID 49910309 Contact Test

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-3142-5762	48h Mortality Rate	100	>100	NA	3.07%		Equal Variance t Two-Sample Test

48h Mortality Rate Summary

C-µg ai/bee	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	6	0	0	0	0	0	0	0		
0	Negative Control	6	0.0167	0	0.0595	0	0.1	0.0167	0.0408	245.0%	
100		6	0	0	0	0	0	0	0		

48h Mortality Rate Detail

C-µg ai/bee	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Solvent Blank	0	0	0	0	0	0
0	Negative Control	0	0	0.1	0	0	0
100		0	0	0	0	0	0

CETIS Analytical Report

OCSPP 850.3020 Acute Honey Bee Test **Huntingdon Life Sciences**

Analysis ID:	14-3781-6506	**Endpoint:**	48h Mortality Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Apr-17 13:15	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	19-2313-5757	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	12 Aug-04	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Reverse Osmosis Water
Ending Date:	27 Apr-17 12:34	**Species:**	Apis mellifera	**Brine:**	
Duration:	4641d 13h	**Source:**	Fowlmere Apiaries	**Age:**	

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	100µg ai/bee passed 48h mortality rate	3.02%

Equal Variance t Two-Sample Test

Control	vs	Conc-µg ai/b	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		100	-1	1.812	0.030	10	CDF	0.8296	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0008333	0.0008333	1	1	0.3409	Non-Significant Effect
Error	0.0083333	0.0008333	10			
Total	0.0091667		11			

Distributional Tests

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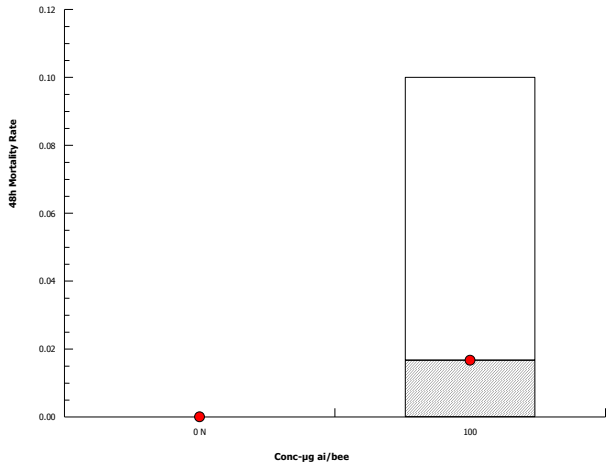
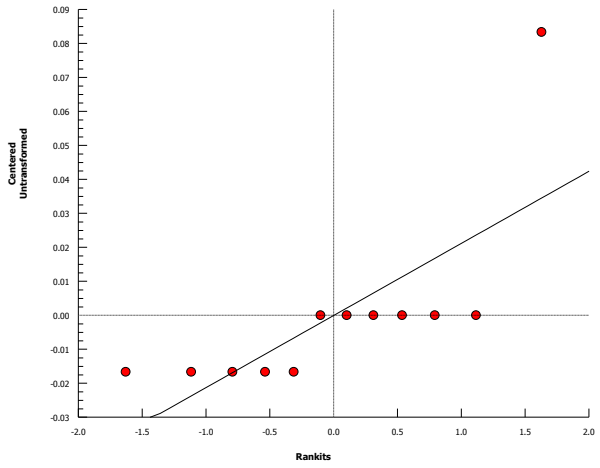
Variances	Mod Levene Equality of Variance Test	1	10.04	0.3409	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.5612	0.8025	5.2E-05	Non-Normal Distribution

48h Mortality Rate Summary

Conc-µg ai/bee	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	6	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
100		6	0.0167	0.0000	0.0595	0.0000	0.0000	0.1000	0.0167	244.95%	1.67%

Graphics





CETIS Summary Report

OCSPP 850.3020 Acute Honey Bee Test **Huntingdon Life Sciences**

Batch ID:	08-7679-1144	**Test Type:**	Mortality (48-h)	**Analyst:**	
Start Date:	12 Aug-04	**Protocol:**	OCSPP 850.3020 Acute Honey Bee	**Diluent:**	Reverse Osmosis Water
Ending Date:		**Species:**	Apis mellifera	**Brine:**	
Duration:	NA	**Source:**	Fowlmere Apiaries	**Age:**	
Sample ID:	02-1544-3611	**Code:**	49910309	**Client:**	CDM Smith - E. Krupka
Sample Date:	12 Aug-04	**Material:**	Ipconazole	**Project:**	Fungicide
Receive Date:		**Source:**	Kureha Corporation		
Sample Age:	NA	**Station:**			

Batch Note: PC Code 125618 MRID 49910309 48h Mortality Rate' endpoint… Error with Log-Normal (Probit) Model: The model requires two or more partial responses.

Sample Note: PC Code 125618 MRID 49910309 Contact Test

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-3142-5762	48h Mortality Rate	100	>100	NA	3.07%		Equal Variance t Two-Sample Test

48h Mortality Rate Summary

C-µg ai/bee	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	6	0	0	0	0	0	0	0		
0	Negative Control	6	0.0167	0	0.0595	0	0.1	0.0167	0.0408	245.0%	
100		6	0	0	0	0	0	0	0		

48h Mortality Rate Detail

C-µg ai/bee	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Solvent Blank	0	0	0	0	0	0
0	Negative Control	0	0	0.1	0	0	0
100		0	0	0	0	0	0